Expression profile of toll-like receptors within the gastrointestinal tract of 2-day-old *Salmonella enteriditis*-infected broiler chickens

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A R T I C L E   I N F O

A B S T R A C T

Salmonella enterica serovar Enteriditis (SE) causes a majority of foodborne illness in the U.S. A more productive avian innate immune response could reduce bacterial colonization and the incidence of infection in humans. However, quantification and comparison of the toll-like receptors (TLR), a component of the innate immune system that recognize bacterial pathogens, and their response to SE colonization across the avian gastrointestinal (GI) tract has not been reported. Therefore, we assessed these changes using real-time qRT-PCR to measure expression of TLR 1LA, 2A, 2B, 3, 4, 5, 7, 15, and 21 in the duodenum, jejunum, ileum, cecal tonsil, ceca, and large intestine of uninfected and SE-infected 2-day-old broiler chickens. Samples were collected soon after hatch to approximate natural SE exposure and to measure initial changes in the immune response to infection. All TLRs had measurable expression within the duodenum, jejunum, ileum, cecal tonsil, ceca, and large intestine. The general expression pattern, with the exception of TLR 21, showed distal GI segments had higher TLR mRNA expression than proximal segments. Infected chickens had increased expression of TLR 1LA, 2A, 4, and 15 in distal GI segments and upregulation of TLR 2B, 3, and 15 in proximal segments, including the duodenum. Interestingly, SE-infection caused downregulation of TLR 5, with no change in TLR 7 or 21. Overall, we provide a comprehensive report of mRNA expression profiles for the TLR family of innate immune receptors in the GI tract of 2-day-old broilers and their differential response to SE colonization.

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1. Introduction

The gram-negative bacteria, *Salmonella enterica* serovar Enteriditis (SE), causes a majority of foodborne illness in the U.S, and the number of cases has remained stable over the last 5 years (2008). The incidence of human infection can be limited by decreasing bacteria in poultry through the induction of avian immune mechanisms. Unfortunately, there is limited information on the function and distribution of many of the components comprising the avian immune response.

In young chickens the innate immune response is particularly important since the acquired response does not fully develop until 1 week of age (Bar-Shira et al., 2003). A key component of the innate response is the pattern recognition receptors (PRRs), particularly the toll-like receptors (TLR) which recognize highly conserved structural motifs of certain microbes (Werling and Coffey, 2007). The following TLRs and their agonists are identified in chickens and are the focus of multiple reviews: TLR 1LA (also reported as 1 type 1, 1/6/10, and16) forms a dimer with TLR 2B; TLR 1LB forms a dimer with both TLR 2's; TLR 2A, peptidoglycan and bacterial lipopeptides; TLR 2B, bacterial lipopeptides and lipopolysaccharide (LPS); TLR 3, double-stranded RNA and poly I:C; TLR 4, LPS; TLR 5, bacterial flagellin; TLR 7, possibly single-stranded RNA; TLR 8, is disrupted and has no known function; TLR 15,

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unique to avian species and upregulated in response to SE-infection; and TLR 21, unknown function and unique to avian and fish species (Fukui et al., 2001; Higgs et al., 2006; Higuchi et al., 2008; Kaiser, 2007; Keestra et al., 2007; Philbin et al., 2005; Schwarz et al., 2007; Werling and Coffey, 2007).

Although TLR agonists have been shown to stimulate the avian innate immune response, we know little about the relative distribution of TLR expression in the gastro-intestinal (GI) tract, which can affect bacteria upon ingestion, colonization, and excretion, and the effect of SE colonization on expression of these genes. Since chickens naturally become infected with bacteria soon after hatch and the innate immune response has a large impact on the level of infection, we chose to assess 2-day-old chickens, after 24 h of SE-infection. We used these birds to establish mRNA expression levels of avian TLRs in GI segments from the duodenum to the large intestine and determine the effect of SE-infection on TLR expression within these segments.

2. Materials and methods

2.1. Experimental animals

Experiments were conducted according to the regulations established by USDA animal care and use committee. Broiler chickens used in this study were obtained from a commercial breeder and were all of the same genetic background. Fertilized eggs were set in incubators (Jamesway Incubator Company, Inc., Ontario, Canada) and maintained at wet and dry bulb temperatures of 32.2 and 37.8 °C, respectively. After 10 days of incubation, the eggs were candled; non-fertile and non-viable eggs were discarded. The viable eggs were returned to the incubator until day 18, when they were transferred to hatchers (Natureform) and maintained under the same temperature and humidity conditions until hatch. At hatch, chickens were placed in floor pens containing wood shavings, provided supplemental heat and were given *ad libitum* access to water and a balanced, unmedicated corn and soybean meal-based chicken starter diet that met or exceeded the levels of critical nutrients recommended by the National Research Council (National Research Council (U.S.), Subcommittee on Poultry Nutrition and NetLibrary Inc., 1994). Twelve animals from one hatch and six animals from a second hatch were obtained at 1 day of age, divided equally into two groups, and placed in either experimental or control rooms at 1 day of age. Experimental animals were then orally dosed with SE and control animals were dosed with a phosphate-buffered saline (PBS) as described below.

2.2. Bacteria

A primary poultry isolate of SE (phage type 13A) from the National Veterinary Services Laboratory (Ames, IA), selected for resistance to novobiocin (NO) and nalidixic acid (NA) in the USDA-ARS facility (College Station, TX) was used. The SE was passed three times into 100 mL of tryptic soy broth (TSB; Difco Laboratories, Detroit, MI) containing 25 μg/mL NO and 20 μg/mL NA (Sigma Chemical Co., St. Louis, MO). The challenge inoculum was cultured in 100 mL of TSB containing antibiotics overnight at 41 °C. Bacteria were washed three times by pelleting at $10,000 \times g$ for 10 min, removing the supernatant, and resuspending in PBS. The concentration of bacteria was determined spectrophoto-metrically at a wavelength of $\lambda = 625$ nm. A stock solution of 1×10^5 cfu/mL was prepared in PBS and 0.5 mL administered orally (5×10^4 cfu/chicken) using an 18-gauge gavage needle. The viable cell concentration of the challenge dose was confirmed by colony counts on Xilose Lisina Tergitol-4 (XLT; Difco Laboratories, Detroit, MI) plates containing NO and NA.

2.3. Sample collection

Control and SE-infected chickens were euthanized 24 h post-challenge. The entire GI tract was removed aseptically and a 25 mg piece of tissue was removed from the middle of the duodenum, jejunum, ileum, ceca, and large intestine and the cecal tonsils. The tissue was washed in PBS and placed in a 2 mL microcentrifuge tube with 1 mL of RNAlater® (QIAGEN Inc., Valencia, CA) and stored at −20 °C until processed.

The liver and spleen were extracted and cultured in an enrichment tetrathionate broth overnight (18 h) at 41 °C to confirm that the control animals were uninfected and the challenged animals were infected with SE. After incubation, the broth was streaked on XLT plates containing NO and NA and incubated for 24 h at 41 °C. The plates were examined for the presence of NO and NA-resistant SE colonies.

2.4. RNA isolation and real-time quantitative RT-PCR

Tissues were removed from RNAlater® and processed using the RNeasy mini kit (QIAGEN Inc.) according to manufacturer's protocol, where tissues were placed in 600 μL of buffer RLT, homogenized using a hand held TissueRuptor (QIAGEN Inc.), and total RNA was eluted in 50 μL of DNase-free water and stored at −80 °C. RNA was quantified using a spectrophotometer (NanoDrop products, Wilmington, DE), and 10 μg of RNA from each sample was treated for 30 min with 1 μL TURBO DNase™ (Ambion, Austin, TX). Samples were evaluated on the Agilent 2100 bioanalyzer (Agilent Technologies, Wilmington, DE) and had no DNA contamination and were of high quality, with an RNA integrity number (RIN) > 8. DNase-treated-total RNA (2 μg) was reverse transcribed using an AffinityScript™ QPCR cDNA synthesis kit (Strategene, La Jolla, CA). The reaction was incubated at 25 °C for 5 min, 42 °C for 30 min, and 95 °C for 5 min. The sample was then diluted in DNase/RNase-free water to 12.5 ng/μL, based on the original RNA concentration, and stored at −20 °C.

Expression of TLR 1LA, 2A, 2B, 3, 4, 5, 7, 15, 21 and a housekeeping gene, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), were determined in GI tissues by real-time quantitative RT-PCR using the MX3000P® QPCR system and MXPro 3.0 software (Strategene, La Jolla, CA). Previous studies in mammals show levels of gene expression typically have a high level of correlation

Table 1
Real-time RT-PCR primer and probe sequences and Genbank accession numbers of the target genes.

Target gene	Forward primer (5′–3′)	Reverse primer (5′–3′)	TAQman probe (5′–3′)	Genbank accession
GAPDH	GGGCACGCCATCACTATCTTC	ACCTGCATCTGCCCATTTGAT	CAGGAGCGTGACCCC	NM_204305
TLR 1LA	GCTTGACTTTAGTGCCTTCATGTTT	GCAAGCAATTGGCAGTAAGCT	AAATGTTATTGTGAGATAAATCT	NM_001007488
TLR 2A	CGCTTAGGAGAGACAATCTGTGAA	AGCCTGTTTTAGGGATTTCAGAGAATTT	ATGCATGGCCTTCTTTAC	NM_204278
TLR 2B	CATGCAAACTTTCACAACACCTTCT	GAATGCTCCAAACTCTGATCTCCAA	CCTTAGCGCAAATTTA	XM_001232192
TLR 3	GCAACACTTCATTGAATAGCCTTGAT	TTCAGTATAAGGCCAAACAGATTTCCA	ATCCTGTGTGAAACTCTT	NM_001011691
TLR 4	GTCCCTGCTGGCAGGAT	TGTCCTGTGCATCTGAAAGCT	CTGGAGGTCATCCCC	NM_001030693
TLR 5	TCACACGGCAATAGTAGCAACA	ACAGGTCACCCAGGTTTGC	CATATTGAATTAGAGAGAAACTG	NM_001024586
TLR 7	TCAGAGGTGGCTGCACAC	CAACAGTGCATTTGACGTCCTT	CATGATGTACCATTTTCC	NM001011688
TLR 15	CCATGCTTCCTGACCTACCTATCT	GTTAAGTTGGAGAGAGATTTAAACCTAGGG	CTCTGCAGCAACTTGAC	NM_001037835
TLR 21	GATGATGGAGACAGCGGAGAAG	GCAGCAGCAGCCAGAGT	CTGGTGCTGGGCCGTG	NM_001030558

(r = 0.8) with protein production when assessed by exon and not by gene (Bitton et al., 2008). All TLR primers and probes were designed using TaqMan® Custom Gene Expression Assays (Applied Biosystem, Austin, TX). Primer amplification efficiency was assessed for each gene using twofold serial dilutions, ranging from 50 to 0.78 ng of transcribed RNA per well. The primer and probe sequences and Genbank accession numbers of the target genes are included in Table 1. RT-PCR was performed for each sample in duplicate in a total volume of 25 μL consisting of 12.5 μL Brilliant® II QPCR Master Mix, 0.5 μL ROX reference dye diluted 1:500, 1.25 μL primer/probe mix (900/250 nM final concentrations, respectively), 8.75 μL RNase/DNase-free water, and 2 μL (25 ng) diluted cDNA. A no template control was included on each plate to ensure there was no contamination. All reaction plates were run under identical cycle conditions, 95 °C for 10 min, and 40 cycles of 95 °C for 30 s, 60 °C for 1 min, and 72 °C for 30 s. The fluorescence threshold was set at 0.2 and the resulting cycle threshold values (C_t), normalized to the reference dye were used for further analysis.

2.5. Statistical analysis

For each gene, infected and control animals were divided equally over two plates with all tissues from one animal included on one plate. For each animal, the difference between the mean C_t value for GAPDH and the target gene was determined and subtracted from 40 to normalize RNA levels between animals and tissues. The $40 - \Delta C_t$ for each sample can be interpreted as a higher numerical value indicating greater gene expression.

All further analyses of $40 - \Delta C_t$ values were performed using the MIXED procedure of SAS version 9.2 (SAS Institute Inc., Cary, NC). Differences in $40 - \Delta C_t$ values across GI segments of uninfected animals were determined from a model with a fixed effect of segment and a random effect of the reaction plate. Differences in $40 - \Delta C_t$ values between SE-infected and uninfected animals within each GI segment were determined from a model with a fixed effect for infection status and a random effect of the reaction plate. The original models included a random effect of hatch, but this was not significant and data were pooled. Values were considered significant at $P < 0.05$, unless stated otherwise. For presentation purposes the least squares means of experimental groups were expressed as fold-changes in gene expression and calculated as $2^{-(x-y)}$, where x is the mean $40 - \Delta C_t$ value of the uninfected group and y is the mean $40 - \Delta C_t$ value of the infected group. However, analysis was not performed on the fold-changes and was only performed on the individual $40 - \Delta C_t$ values.

3. Results

3.1. Experimental infection

The actual challenge dose was determined from the colony counts on XLT plates and was 3×10^4 cfu/bird. Liver and spleen cultures from infected birds were all positive for SE and all negative for uninfected birds.

3.2. Toll-like receptor mRNA expression in the gastrointestinal tract of 2-day-old broilers

All primer and probe pairs were found to have gene amplification efficiencies close to 100% based on the slope of C_t values obtained from serial-diluted cDNA. Gene efficiencies ranged from 98.5 to 104.6%. Toll-like receptor mRNA expression was detected for all genes in all samples analyzed and are presented in Fig. 1. Overall TLR expression was highest for TLR 3 and lowest for TLR 1LA. With the exception of TLR 21, expression of each TLR was highest in the distal segments of the GI tract including the cecal tonsils, ceca, and large intestine (Fig. 1). Gene expression was lowest within the duodenum for all TLRs except TLR 15 and 21, where expression was lowest in the jejunum and cecal tonsil, respectively. The largest mRNA expression differences ($P < 0.05$) were observed between the duodenum and the cecal tonsils for TLR 1LA, 2B, 3, and 7, with 2.4, 2.3, 3.3, and 3.8-fold higher cecal tonsil expression, respectively. Differences were largest ($P < 0.05$) between the duodenum and ceca for TLR 2A, 4, and 5, with 4.9, 2.6, and 2.6-fold higher ceca expression, respectively. The jejunum and cecal tonsil segments had the greatest differences in mRNA expression for TLR 15 (3-fold higher in the cecal tonsils, $P = 0.001$) and TLR 21 (2.9-fold lower in the cecal tonsils, $P = 0.01$).

Expression of most TLRs in uninfected birds had non-significant differences within distal GI segments, except TLR 2A, 3, and 5 (Fig. 1). TLR 2A expression was higher (1.6-fold) in the ceca compared to the cecal tonsils and the large
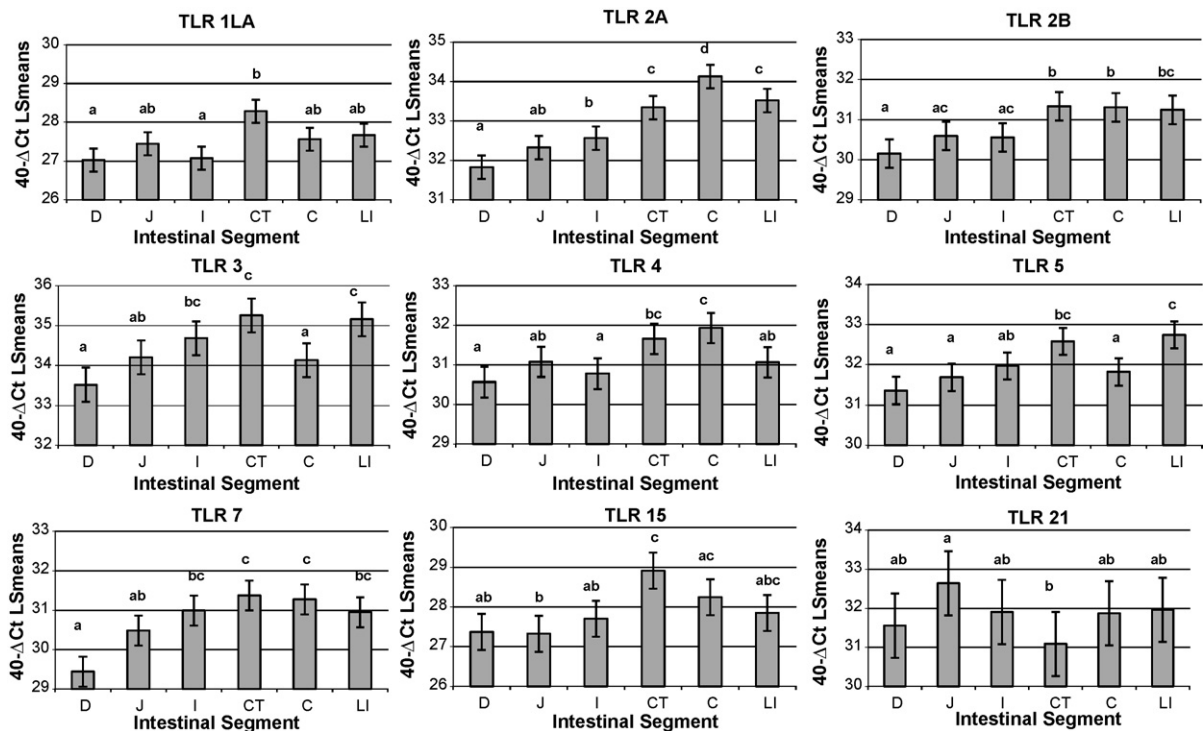
Fig. 1. Differential expression of toll-like receptor (TLR) 1LA, 2A, 2B, 3, 4, 5, 7, 15, and 21 mRNA in the duodenum (D), jejunum (J), ileum (I), cecal tonsil (CT), ceca (C), large intestine (LI) of 2-day-old broilers. GAPDH was used as an internal control for real-time qRT-PCR analysis. The data are from nine individual animals over two hatches. Columns with different letters have significantly different ($P < 0.05$) $40 - \Delta C_t$ least squares means. Error bars indicate the standard error of the least squares means.

intestine. However, decreased expression was observed for both TLR 3 (2.1-fold) and TLR 5 (1.8-fold) in the ceca compared to both the cecal tonsils and large intestine.

3.3. Expression of toll-like receptor mRNA in the gastrointestinal tract of broilers following SE-infection

Infection resulted in significant expression differences for all TLRs in at least one segment of the GI tract, with the exception of the anti-viral receptor, TLR 7, and a receptor with unknown ligand, TLR 21 (Fig. 2). In segments with significant differences, almost all had upregulated TLR mRNA expression after SE-infection (Table 2). The exception was TLR 5, with a twofold ($P = 0.05$) and threefold ($P = 0.01$) decrease in expression after infection within the cecal tonsils and ceca, respectively. Although SE does not colonize the duodenum to any great extent (Fanelli et al., 1971), increased expression of TLR 2B, 3, and 15 was found in this segment. Both TLR 2B and 3 had the greatest changes in expression in the proximal GI tract, whereas the greatest upregulation of TLR 1LA, 2A, 4, 5, and 15 occurred in the distal intestinal segments. An increase in TLR 15 mRNA expression was observed in all intestinal segments. The most dramatic increase was in TLR 15 expression within the ceca of infected birds, increasing 20-fold ($P < 0.001$) compared to uninfected chickens. However, the ileum experienced changes in the greatest number of TLRs, including TLR 1LA, 2A, 2B, 3, and 15.

Table 2

Fold-changes in mRNA expression of toll-like receptors (TLR) within the gastrointestinal tract of 2-day-old *Salmonella enterica* serovar Enteriditis-infected broiler chickens. Data are expressed as $2^{-\Delta\Delta C_t}$. Positive values indicate infected animals have an increase in mRNA expression compared to uninfected animals. Significant differences are indicated with $^*P < 0.05$ and $^{**}P < 0.01$. Statistical analysis was performed on ΔC_t values. D, duodenum; J, jejunum; I, ileum; CT, cecal tonsil; C, ceca; LI, large intestine.

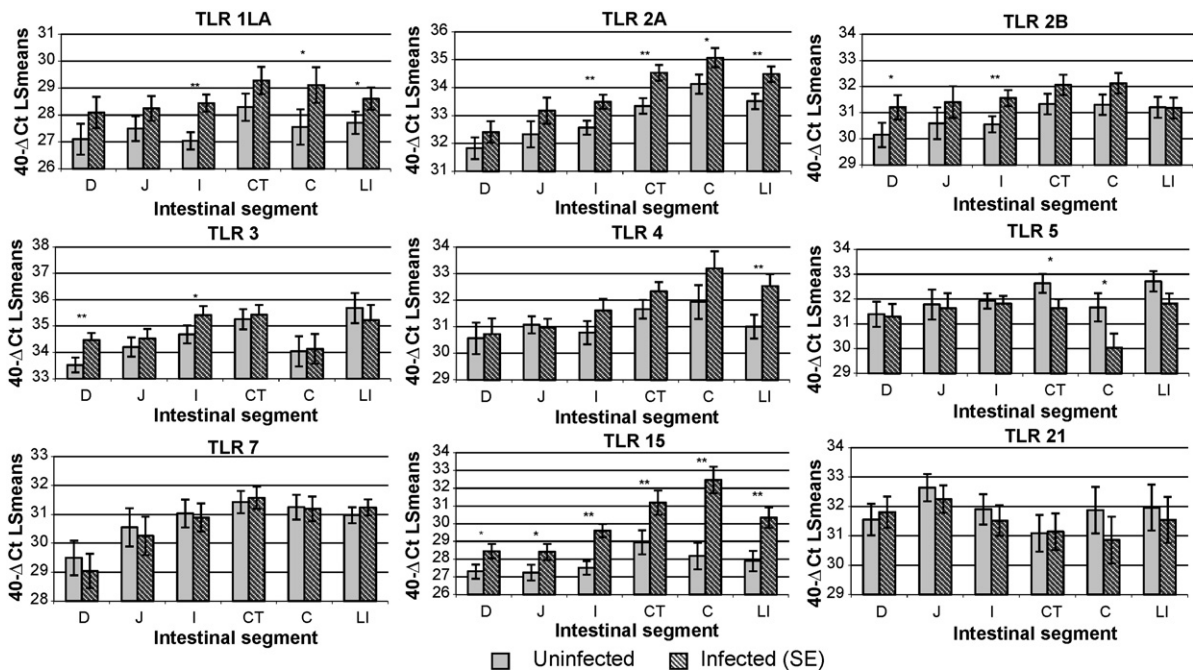|	D	J	I	CT	C	LI
TLR 1LA	2.00	1.69	**2.64****	1.99	**2.95***	**1.86***
TLR 2A	1.50	1.80	**1.90****	**2.28****	**1.92***	**1.97****
TLR 2B	**2.08***	1.75	**2.01****	1.66	1.77	−1.02
TLR 3	**1.92****	1.25	**1.67***	1.12	1.07	−1.37
TLR 4	1.12	−1.07	1.79	1.60	2.40	**2.89****
TLR 5	−1.07	−1.10	−1.08	**−2.01***	**−3.08***	−1.85
TLR 7	−1.37	−1.23	−1.10	1.11	−1.04	1.20
TLR 15	**2.21***	**2.25***	**4.28****	**4.74****	**19.54****	**5.46****
TLR 21	1.19	−1.31	−1.30	1.04	−2.02	−1.33

Fig. 2. Differential expression of toll-like receptor (TLR) 1LA, 2A 2B, 3, 4, 5, 7, 15, and 21 mRNA in the duodenum (D), jejunum (J), ileum (I), cecal tonsil (CT), ceca (C), large intestine (LI) of uninfected chickens or *Salmonella enterica* serovar Enteriditis (SE) chickens infected for 24 h. GAPDH was used as an internal control for real-time qRT-PCR analysis. The data are from nine individual animals over two hatches. Infected and uninfected $40 - \Delta C_t$ least squares means differ at $^*P < 0.05$, $^{**}P < 0.01$. Error bars indicate the standard error of the least squares means.

4. Discussion

Toll-like receptors have been studied to a great extent in mice and humans, demonstrating the necessity of these receptors in reduction and clearance of bacterial pathogens (Albiger et al., 2007), but limited information is available in chickens. We provide the first assessment of quantifiable changes in TLR expression across different segments of the GI tract and alterations in TLR expression within the first 24 h of SE-infection in 2-day-old broiler chickens.

Within all segments of the GI tract, we found measurable mRNA expression for each TLR. Iqbal et al. (2005) reported the presence of TLR 1LA, 2B, 3, 4, 5, and 7 mRNA, but found little to no TLR 2A mRNA in the same tissues of 8-week-old Leghorns. Cecal expression of TLR 2, 4, and 5 was also reported in 1-week-old chickens from F8 advanced intercross lines (Abasht et al., 2008). Ceca of 2-day-old infected broilers express TLR 15, but little to no expression is found in the small intestine of 5-week-old broilers (Higgs et al., 2006). The greater initial TLR expression in younger birds indicates the potential for a stronger innate immune response that may limit bacterial infection during this time period. Overall, our results support the presence of previously reported TLR mRNA's in chicken GI segments analyzed, and we have shown for the first time that TLR 2A, 15, and 21 mRNA are present in tissues from the duodenum to the large intestine.

We found overall expression of TLR 1LA was lower than other TLRs and similar across most GI segments of uninfected birds. However, infection upregulated TLR 1LA in the ceca (3-fold) and the ileum (2.6-fold), with a similar expression pattern observed for TLR 2B before and after infection. In agreement with reports of similar (2.3-fold, Abasht et al., 2008) and slightly greater (4.5-fold, Higgs et al., 2006) increases in TLR 2 (both A and B) expression in ceca of infected broilers, we found a twofold upregulation of TLR 2B in the proximal GI after infection. Dimerization of these two receptors is needed for the recognition of bacterial lipoprotein and LPS (Fukui et al., 2001; Higuchi et al., 2008; Keestra et al., 2007). Increased expression of both receptors in the ileum may play a role in limiting retrograde movement of bacteria to nutrient absorbing areas of the GI tract.

Uninfected birds had high expression of TLR 2A compared to other TLRs, which increased from the duodenum to the ceca and remained high in the large intestine. Infection upregulated TLR 2A (twofold) in the ileum and distal GI segments, facilitating increased recognition of lipoproteins and peptidoglycan. These results support *in vitro* results of increased TLR 2 expression in lipoprotein- and LPS-stimulated, transfected HEK293 cells (van Aubel et al., 2007). A high degree of resemblance was observed between the expression pattern of TLR 2A, 4 and 15 in infected animals. The similarity was also observed for TLR 2 and 4 (Abasht et al., 2008) and TLR 2 and 15 (Higgs et al., 2006) in other studies, indicating possible dimerization or cross-regulation.

Recognition of LPS occurs through engagement with TLR 4, and possibly TLR 2, and is linked with susceptibility to *Salmonella* infection in chickens (Leveque et al., 2003). In contrast to results reported by Abasht et al. (2008) and in agreement with Higgs et al. (2006), we did not find TLR 4 to

increase significantly in the ceca after infection. The differences observed between studies may result from difference in length of infection (1 day versus 7 days). Studies in infected mice show expression of TLR 4 decreases at 24 h but increases at time points thereafter (Totemeyer et al., 2003). Chicken embryonic fibroblasts stimulated with heat-killed *Salmonella* increase TLR 4 expression at 6 h, but return to control levels by 24 h (Higgs et al., 2006). Our results may be measuring TLR 4 after peak expression in the midst of downregulation. However, we found infection increased expression of TLR 4 in the large intestine, which would facilitate reduced fecal shedding of pathogens and limit environmental and processed poultry product contamination.

Decreased expression of TLR 5 compared to other GI segments was found in uninfected birds, with infection causing further downregulation (threefold) in the ceca. Other investigators have found a similar decrease in TLR 5 expression after *Salmonella* infection (Abasht et al., 2008). In human and mouse intestinal epithelium, TLR 5 downregulation is caused by TLR 2 and 4 agonists and IFN-γ (Ortega-Cava et al., 2006; van Aubel et al., 2007). Our observed reduction in ceca and cecal tonsil TLR 5 expression is most likely attributed to negative feedback in avian epithelial cells, which may be needed to limit overstimulation in an area of high bacterial colonization.

TLR 3 is highly expressed in many chicken tissues, but is limited in expression within immune cells and immune tissue (He et al., 2006; Iqbal et al., 2005; Kogut et al., 2005). Although TLR 3 is an anti-viral receptor, we found increased expression during bacterial infection within the duodenum and ileum, possibly creating a more immunocompetent intestine. Overall, the higher level of TLR 3 compared to other TLRs in the GI tract may increase the birds' ability to respond to viral infection due to non-functional TLR 8 and limited functionality of TLR 7 (Schwarz et al., 2007). Although, we did find TLR 7 expression increased from the proximal to the distal intestine and remained unchanged after SE-infection. As TLR 7 is also a receptor for viral ligands, it is not surprising that bacterial infection did not alter its expression. TLR 21 was the only other receptor to remain unaltered by bacterial infection, possibly implicating this receptor in viral recognition. The expression pattern of TLR 21 in uninfected birds was unlike any other TLR with highest expression in the jejunum and lowest expression in the cecal tonsils.

The most substantial changes in gene expression in this study were found for the recently described TLR 15. In agreement with Higgs et al. (2006), we found upregulation of TLR 15 in the intestine of SE-infected chickens. TLR 15 may be preferentially located on immune cell subsets, as higher expression is observed in bone marrow and bursa (Higgs et al., 2006) and our laboratory has found the upregulation of TLR 15 in SE-stimulated heterophils (unpublished data). Therefore, the observed changes in TLR 15 and potentially all other TLRs may be a function of cell infiltration as well as changes in expression of existing cells. We have shown expression of TLR 15 is highly responsive to SE, which may have a large impact on the innate response to SE in young broilers and should be further evaluated.

In summary, we provide the first report of quantifiable comparisons of TLR 1LA, 2A, 2B, 3, 4, 5, 7, 15, and 21 across the GI tract of uninfected birds and changes in expression after 24 h of infection with SE. Although TLR 21 has been identified in the chicken genome, to the best of our knowledge this is the first time mRNA expression has been reported in chickens. In general, our results showed TLR expression was higher in the distal intestinal segments and tended to increase with infection, with the exception of TLR 5, 7, and 21. Although mRNA expression levels do not necessarily correlate with protein levels (Bitton et al., 2008), the data provided indicate potential TLRs that could be modulated to increase resistance to *Salmonella*, most notably TLR 15.

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